

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 1-98)		ATTORNEY'S DOCKET NUMBER T/97300 US
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		
INTERNATIONAL APPLICATION NO. PCT/EP98/04945	INTERNATIONAL FILING DATE 05-AUG-1998	PRIORITY DATE CLAIMED 08-AUG-1997
TITLE OF INVENTION NUCLEIC ACID SEQUENCES THAT CAN BE USED AS PRIMERS AND PROBES IN THE AMPLIFICATION AND DETECTION OF ALL SUBTYPES OF HIV-1		
APPLICANT(S) FOR DO/EO/US Jaap GOUDSMIT; Pieter OUDSHOORN; Suzanne JURRIAANS; Vladimir V. LUKASHOV Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</p> <p>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <ul style="list-style-type: none"> a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <p>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <ul style="list-style-type: none"> a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). b. <input checked="" type="checkbox"/> have been transmitted by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11. to 16. below concern document(s) or information included:</p> <p>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input checked="" type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input checked="" type="checkbox"/> A FIRST preliminary amendment.</p> <p><input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information: Sequence Listing Paper Copy and CRF Diskette</p>		

U.S. APPLICATION NO. (if known, see 37 CFR 1.65) 09/463352	INTERNATIONAL APPLICATION NO PCT/EP98/04945	ATTORNEY'S DOCKET NUMBER T/97300 US
17. <input checked="" type="checkbox"/> The following fees are submitted:		CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO		\$1070.00
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO		\$930.00
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO		\$790.00
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)		\$720.00
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)		\$98.00
ENTER APPROPRIATE BASIC FEE AMOUNT = \$ 840.00		
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		\$
CLAIMS	NUMBER FILED	NUMBER EXTRA
Total claims	9 - 20 =	x \$22.00
Independent claims	1 - 3 =	x \$82.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		+ \$270.00
TOTAL OF ABOVE CALCULATIONS = \$		
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).		+ \$
SUBTOTAL = \$		
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).		\$
TOTAL NATIONAL FEE = \$		
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property		+ \$ 40.00
TOTAL FEES ENCLOSED = \$		
		Amount to be refunded: \$
		charged: \$ 880.00
<p>a. <input type="checkbox"/> A check in the amount of \$ _____ to cover the above fees is enclosed.</p> <p>b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. <u>02-2334</u> in the amount of \$ <u>880.00</u> to cover the above fees. A duplicate copy of this sheet is enclosed.</p> <p>c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-2334</u>. A duplicate copy of this sheet is enclosed.</p>		
<p>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.</p> <p>SEND ALL CORRESPONDENCE TO:</p> <p>Mary E. Gormley 1300 Piccard Drive Suite 206 Rockville, Maryland 20850-4396</p>		
<p><u>Mary E. Gormley</u> SIGNATURE</p> <p>Mary E. Gormley NAME</p> <p>34,409 REGISTRATION NUMBER</p>		

09/463352

420 Rec'd PCT/PTO 21 JAN 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Jaap GOUDSMIT, Pieter OUDSHOORN, Suzanne JURRIAANS,
Vladimir Vladimirovich LUKASHOV

Serial Number: To be assigned Group Art Unit: To be assigned

Filed: Concurrently herewith Examiner: To be assigned

For: NUCLEIC ACID SEQUENCES THAT CAN BE USED AS PRIMERS
AND PROBES IN THE AMPLIFICATION AND DETECTION OF
ALL SUBTYPES OF HIV-1

Corresponding to: PCT/EP98/04945, filed August 5, 1998

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

January 21, 2000

Sir:

Prior to the calculation of the fee in the above-identified application, please enter the following amendments:

IN THE SPECIFICATION:

Page 1, line 3, please insert -- FIELD OF INVENTION -- ; and

between lines 6 and 7, please insert -- BACKGROUND OF THE
INVENTION --.

Page 3, between lines 16 and 17, please insert -- SUMMARY OF THE
INVENTION --; and

between lines 28 and 29, please insert -- DETAILED DESCRIPTION OF
THE INVENTION --.

Page 23, line 1, please change "Claims" to -- We Claim --.

IN THE CLAIMS:

Please amend the claims as follows:

Claim 1, line 1, please change "Pair" to -- A pair --;

line 4, please delete both commas and insert
-- sequential -- before "nucleotides";

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line 6, please insert -- ; -- after “AGA GA”;
line 7, please insert -- ; and -- after “TAG A”;
line 8, please insert -- ; -- after “CGGGCGCCACTGCTA”;
line 9, please delete “,” after “comprising”;
line 10, please insert -- sequential -- before “nucleotides” and
delete the “,” after “nucleotides”;
line 12, please insert -- ; -- after “ATA AA”;
line 13, please delete “.” after “CTT GA” and insert therefor “;”;
line 14, please insert -- . – after “AGT”.

Claim 2, line 1, please change “Pair” to -- The pair --;

line 2, please delete “,” after “comprising”;
line 3, please insert -- sequential -- before “nucleotides”;
line 4, please insert -- ; -- after “AGA GA”;
line 5, please delete “,” after “comprising”; and insert
-- sequential -- before “nucleotides”.

Claim 3, line 1, please change “Pair” to -- The pair --; and delete
“ or 2” and insert therefor -- , -- .

Claim 4, line 1, please change “Pair” to -- The pair -- ; and
insert -- , -- after “claim 3”;

Claim 5, line 1, please change “Method” to -- A method --;
line 3, please delete “any of claims 1-4” and insert therefor
-- claim 1, --;
line 4, please delete “and” after “reagents” and insert therefor
-- , wherein -- .

Claim 6, line 1, please change “Method” to -- The method --;
line 7, please insert -- , -- after “TCT GTT”;
line 8, please change “on” to -- one -- and insert
-- , -- after “label”;
line 9, please insert -- , -- after “conditions”.

Claim 7, line 1, please change “Method” to -- The method --.

Claim 8, line 1, please change “Test” to -- A test --;

line 2, please change "accroding" to -- according --; and
please change the "," to -- ; -- after
"claim 1";
line 5, please insert -- ; and -- after "label".
Claim 9, line 1, please change "Test" to -- A test --, and
insert -- , -- after the word "sample";
line 2, please change "oligonucleotide" to --oligonucleotides --.
Please cancel claim 10, without prejudice or disclaimer of the subject
matter thereof.

REMARKS

The specification and claims 1 - 9 are amended, and claim 10 has been
cancelled, hereby. Claims 1 - 9 are presented for examination.

It is believed that claims 1 - 9 recite a patentable improvement in the art.
Favorable action is solicited. In the event any fees are required with this paper,
please charge our Deposit Account No. 02-2334.

Respectfully submitted,

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Nucleic acid sequences that can be used as primers and probes in the amplification and detection of all subtypes of HIV-1.

The present invention is related to nucleic acid sequences that can be used in the field of 5 virus diagnostics, more specifically the diagnosis of infections with the AIDS causing Human Immuno-deficiency Virus (HIV).

Whereas conventional virus diagnosis has been based predominantly on the detection of viral antigens or specific antibodies thereto, in recent years attention has shifted towards 10 methods for the direct detection of the genome of viruses or nucleic acid sequences derived thereof, both RNA and DNA. These methods are usually based on nucleic acid hybridization.

Nucleic acid hybridization is based on the ability of two strands of nucleic acid containing 15 complementary sequences to anneal to each other under the appropriate conditions, thus forming a double stranded structure. When the complementary strand is labeled, the label can be detected and is indicative for the presence of the target sequence. Especially in combination with methods for the amplification of nucleic acid sequences these methods have become an important tool in viral diagnosis, in particular for the detection of human immunodeficiency virus (HIV).

Nucleic acid amplification techniques are especially useful as an additional technique in cases where serological methods give doubtful results or in cases where there may be a 20 considerable time period between infection and the development of antibodies to the virus. With HIV, seroconversion usually can occur some 3-6 months after exposure to the virus. Thus, whereas no antibodies will be detected with conventional immunoassays, proviral DNA or circulating viral RNA may already be detectable. Also in monitoring antiviral therapy, 25 methods based on nucleic acid amplification have several advantages over serological methods. Especially quantitative amplification methods provide a powerful tool in assessing the changes in the amount of virus present before and during therapy.

The choice of the oligonucleotides to be used as primers and probes in the amplification and detection of nucleic acid sequences is critical for the sensitivity and specificity of the assay.

The sequence to be amplified is usually only present in a sample (for example a blood 30 sample obtained from a patient suspected of having a viral infection) in minute amounts. The primers should be sufficiently complementary to the target sequence to allow efficient amplification of the viral nucleic acid present in the sample. If the primers do not anneal properly (due to mispairing of the bases on the nucleotides in both strands) to the target sequence, amplification is seriously hampered. This will effect the sensitivity of the assay 35 and may result in false negative test results. Due to the heterogeneity of viral genomes false negative test results may be obtained if the primers and probes are capable of recognizing sequences present in only part of the variants of the virus. The HIV virus shows a high

heterogeneity. Genetic variability has been demonstrated amongst isolates from different continents but also between individuals and between different stages of the disease. Based on sequence analysis two groups within HIV-1 have been identified: group M (M for "major"), and group O (O for "outlier"). Within group M subtypes (A-H), each constituting a

5 phylogenetic separate set of sequences, have been assigned and additional ones are being identified. This sequence variation is not uniformly distributed throughout the genome. The HIV-1 genome, like all retroviral genomes, roughly consists of the following regions: The *gag* gene of the HIV-1 genome is the region encoding the core proteins of the virus (for example, p24). The *env* gene encodes a large precursor protein, gp160, which is processed into the
10 envelop proteins gp 120 and gp41. The *pol* gene encodes the polymerase of the virus (reverse transcriptase). The Long Terminal Repeat region's (LTR's) are the regions on the viral genome that participate in the integration of the virus with the host cell and in the regulation of transcription of the viral genes. Some regions are more prone to sequence variation than others. Especially in the *env* domain sequence variation can be as high as
15 30% between members of the different subtypes. Ideally, primer selection should be based on knowledge of interstrain variability in candidate primer sequences and the consequences of mismatching at primer sites. McCutchan et al, J.AIDS, 4, 1241-1250, 1991, used PCR to make a genetic comparison of different HIV-1 isolates. Using anchored PCR (varying sense primers were used with a constant antisense primer. primers were chosen from relatively
20 conserved regions in *gag*, *env* and LTR) The effect of primer mispairing on the amount of PCR product obtained was also investigated. Mispairing at the 3' end of the primer decreased the amount of product sometimes more then 100-fold.

The detection of all presently known subtypes of HIV-1 is of extreme importance, especially with regard to patient management, security of blood and blood products and clinical- and
25 epidemiological studies. Current assays for the amplification and subsequent detection of HIV-1 derived nucleic acid sequences are usually based on amplification of sequences in the *gag* region of the viral genome. These assays have been developed for subtype B, which is the major subtype in European countries and the United States. However, the presence of other subtypes, which were geographically confined before, is increasing due to
30 frequent travel between these countries and, for example, African countries. Sensitive assays are therefore needed that are capable of detecting as much variants of the HIV-1 virus as possible (preferably all).

Research aimed at identifying suitable primer sets for the reliable amplification of HIV-1 derived nucleic acid sequences has been ongoing for the past years. Engelbrecht et al.,
35 J.Virol.Meth., 55, 391-400, 1995, describe a study aimed at the development of a specific and sensitive PCR protocol using *env*, *gag* and LTR primer pairs to detect subtypes present

in the Western Cape, South Africa. Twenty four strains of which it was known that they belonged to subtypes B, C and D were analyzed. It was found that the performance of the primer pairs was greatly dependent on the optimization of the reaction conditions for the different primer pairs. Only when less stringent conditions were used (for example, with the

5 LTR primer pair an increased cycle time and lower annealing temperatures were required) these particular strains of HIV could be detected with sufficient sensitivity and reproducibility with all primer pairs.

Zazzi et al., J. Med. Virol., 38, 172-174, 1992, developed a two-step PCR reaction (using nested primers) for the detection of HIV-1 DNA in clinical samples. The primers used for
10 amplification were derived from the gag gene and the LTR region. The patients tested in this study were all from neighbouring areas, which makes it likely that they represent only a limited number of different viral strains.

A quantitative PCR method using LTR derived nested primers was described by Vener et al. in BioTechniques, 21, 248-255, 1996. This procedure was only tested on HIV-1_{MN} infected
15 peripheral blood mononuclear cells (PBMC). Thus nothing can be said about the suitability of the primers used for detecting different subtypes of the virus.

With the present invention nucleotide sequences are provided that can be used as primers and probes in the amplification and detection of HIV-1 nucleic acid. The oligonucleotide sequences provided with the present invention are located in the LTR part of the HIV viral
20 genome. It has been found that, by using the sequences of the present invention in methods for the amplification and detection of nucleic acid a sensitive and specific detection of HIV-1 can be obtained. The benefit of the sequences of the present invention primarily resides in the fact that, with the aid of primers and probes comprising the sequences according to the invention the nucleic acid of all presently known subtypes of HIV-1 can be detected with high
25 accuracy and sensitivity. So far no primer pairs or hybridization probes have been developed that would allow the detection of such a broad range of HIV-1 variants.

The oligonucleotide sequences according to the present invention are especially useful in methods for the amplification of nucleic acid.

Various techniques for amplifying nucleic acid are known in the art. One example of a
30 technique for the amplification of a DNA target segment is the so-called "polymerase chain reaction" (PCR). With the PCR technique the copy number of a particular target segment is increased exponentially with a number of cycles. A pair of primers is used and in each cycle a DNA primer is annealed to the 3' side of each of the two strands of the double stranded DNA-target sequence. The primers are extended with a DNA polymerase in the presence of
35 the various mononucleotides to generate double stranded DNA again. The strands of the double stranded DNA are separated from each other by thermal denaturation and each

strand serves as a template for primer annealing and subsequent elongation in a following cycle. The PCR method has been described in Saiki *et al.*, *Science* 230, 135, 1985 and in European Patents no. EP 200362 and EP 201184.

Another technique for the amplification of nucleic acid is the so-called transcription based amplification system (TAS). The TAS method is described in International Patent Appl. no. WO 88/10315. Transcription based amplification techniques usually comprise treating target nucleic acid with two oligonucleotides one of which comprises a promoter sequence, to generate a template including a functional promoter. Multiple copies of RNA are transcribed from said template and can serve as a basis for further amplification.

10 An isothermal continuous transcription based amplification method is the so-called NASBA process ("NASBA") as described in European Patent no. EP 329822. NASBA includes the use of T7 RNA polymerase to transcribe multiple copies of RNA from a template including a T7 promoter. Other transcription based amplification techniques are described in EP 408295. EP 408295 is primarily concerned with a two-enzyme transcription based

15 amplification method. Transcription based amplification methods, such as the NASBA method as described in EP 329822, are usually employed with a set of oligonucleotides, one of which is provided with a promoter sequence that is recognized by a DNA dependent RNA polymerase such as, for example, T7 polymerase. Several modifications of transcription based techniques are known in the art. These modifications comprise, for example, the use of blocked oligonucleotides (that may be provided with a promoter sequence). These oligo's are blocked so as to inhibit an extension reaction proceeding therefrom (US5554516). One or more "promoter-primers" (oligonucleotides provided with a promoter sequence) may be used in transcription based amplification techniques, optionally combined with the use of one or more oligonucleotides that are not provided with a promoter sequence. For RNA

25 amplification, a transcription based amplification technique, is a preferred technology.

Amplification using PCR, can also be based on an RNA template. The actual PCR needs to be preceded by a reverse transcription step to copy the RNA into DNA (RT-PCR). However, if RT-PCR is used for the detection of viral transcripts differentiation of mRNA- and DNA-derived PCR products is necessary. DNase treatment prior to RT-PCR can be employed

30 (Bitsch, A. *et al.*, *J Infect. Dis* 167, 740-743., 1993; Meyer, T. *et al.*, *Mol. Cell Probes*. 8, 261-271., 1994), but sometimes fails to remove contaminating DNA sufficiently (Bitsch, A. *et al.*, 1993).

In contrast to RT-PCR, NASBA, which is based on RNA transcription by T7 RNA polymerase (Kievits *et al.*, 1991; Compton, 1991), does not need differentiation between RNA- and DNA-derived amplification products since it uses RNA as its principal target. NASBA enables specific amplification of RNA targets even in a background of DNA.

The use of the oligonucleotides according to the invention is not limited to any particular amplification technique or any particular modification thereof. It is evident that the oligonucleotides according to the invention find their use in many different nucleic acid amplification techniques and various methods for detecting the presence of (amplified) nucleic acid of HIV. The oligonucleotides of the present invention can likewise be used in quantitative amplification methods. An example if such quantitative method is described in EP 525882.

5 The term "oligonucleotide" as used herein refers to a molecule comprised of two or more deoxyribonucleotides or ribonucleotides. Such oligonucleotides may be used as primers and probes.

10 Of course, based on the sequences of the oligonucleotides of the present invention, analogues of oligonucleotides can also be prepared. Such analogues may constitute alternative structures such as "PNA" (molecules with a peptide-like backbone instead of the phosphate sugar backbone of normal nucleic acid) or the like. It is evident that these 15 alternative structures, representing the sequences of the present invention are likewise part of the present invention.

15 The term "primer" as used herein refers to an oligonucleotide either naturally occurring (e.g. as a restriction fragment) or produced synthetically, which is capable of acting as a point of initiation of synthesis of a primer extension product which is complementary to a nucleic acid 20 strand (template or target sequence) when placed under suitable conditions (e.g. buffer, salt, temperature and pH) in the presence of nucleotides and an agent for nucleic acid polymerization, such as DNA dependent or RNA dependent polymerase. A primer must be sufficiently long to prime the synthesis of extension products in the presence of an agent for 25 polymerization. A typical primer contains at least about 10 nucleotides in length of a sequence substantially complementary or homologous to the target sequence, but somewhat longer primers are preferred. Usually primers contain about 15-26 nucleotides but longer primers may also be employed, especially when the primers contain additional sequences such as a promoter sequence for a particular polymerase.

25 Normally a set of primers will consist of at least two primers, one 'upstream' and one 30 'downstream' primer which together define the amplicate (the sequence that will be amplified using said primers).

Primarily for the use in transcription based amplification techniques, the oligonucleotides according to the invention may also be linked to a promoter sequence. The term "promoter sequence" defines a region of a nucleic acid sequence that is specifically recognized by an 35 RNA polymerase that binds to a recognized sequence and initiates the process of transcription by which an RNA transcript is produced. In principle any promoter sequence

may be employed for which there is a known and available polymerase that is capable of recognizing the initiation sequence. Known and useful promoters are those that are recognized by certain bacteriophage RNA polymerases such as bacteriophage T3, T7 or SP6. Oligonucleotides linked to a promoter sequence are commonly referred to as "promoter primers". Their function as a primer, e.g. the starting point for an elongation reaction, however, may be blocked, as already mentioned above, or absent in some embodiments of transcription based amplification reactions.

An oligonucleotide according to the present invention is substantially complementary to a sequence of the LTR region of a nucleic acid sequence of a HIV genome, said

10 oligonucleotide being 10-50 nucleotides in length and comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 1: G GGC GCC ACT GCT AGA GA

SEQ ID 2: G TTC GGG CGC CAC TGC TAG A

SEQ ID 3: CGGGCGCCACTGCTA

15 SEQ ID 4: CTG CTT AAA GCC TCA ATA AA

SEQ ID 5: CTC AAT AAA GCT TGC CTT GA

SEQ ID 6: TCT GGT AAC TAG AGA TCC CTC

SEQ ID 7: TAG TGT GTG CCC GTC TGT

SEQ ID 8: AGT GTG TGC CCG TCT GTT

20 SEQ ID 12: GAT GCA TGC TCA ATA AAG CTT GCC TTG AGT

or the complementary sequence thereof.

It is understood that oligonucleotides consisting of the sequences of the present invention may contain minor deletions, additions and/or substitutions of nucleic acid bases, to the extent that such alterations do not negatively affect the yield or product obtained to a

25 significant degree. Where oligonucleotides according to the present invention are used as probes, the alterations should not result in lowering the hybridization efficiency of the probe.

For example, in case of transcription based amplification techniques, wherein one or more of the primers may be provided with a promoter sequence, the introduction of a purine-rich (= G or A) hybridizing sequence, just after the promoter sequence may have positive effects on

30 the transcription (when there are C's and T's abortive transcription may occur). If no such sequence is available in the target nucleic acid a purine-rich sequence can be inserted in the oligonucleotide just following the last three G residues of the promoter sequence.

The sequences of the present invention are reflected as DNA sequences. The RNA equivalents of these sequences are likewise part of the present invention.

35 Preferred oligonucleotides according to the invention are oligonucleotides consisting essentially of a sequence selected from the group consisting of:

SEQ ID 1: G GGC GCC ACT GCT AGA GA

SEQ ID 2: G TTC GGG CGC CAC TGC TAG A

SEQ ID 3: CGGGCGCCACTGCTA

SEQ ID 4: CTG CTT AAA GCC TCA ATA AA

5 SEQ ID 5: CTC AAT AAA GCT TGC CTT GA

SEQ ID 6: TCT GGT AAC TAG AGA TCC CTC

SEQ ID 7: TAG TGT GTG CCC GTC TGT.

SEQ ID 8: AGT GTG TGC CCG TCT GTT.

SEQ ID 9: *aat tct aat acg act cac tat agg g*AG AGG GGC GCC ACT GCT AGA GA

10 SEQ ID 10: *aat tct aat acg act cac tat agg g*AG AGG TTC GGG CGC CAC TGC TAG A

SEQ ID 11: *aat tct aat acg act cac tat agg g*CGGGCGCCACTGCTA

SEQ ID 12: GAT GCA TGC TCA ATA AAG CTT GCC TTG AGT

SEQ ID 9-11 actually comprise the sequence as reflected by SEQ ID 1-3. In SEQ ID 9-11,

the sequences of SEQ ID 1-3 are operably linked to a promoter sequence (the T7 promoter sequence). This makes the sequences especially suitable for use as upstream primer in a transcription based amplification technique such as NASBA.

A preferred embodiment of the present invention is a combination of two oligonucleotides according to the invention, for use as a set in nucleic acid amplification.

Such a pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the LTR region of the genome of HIV-1, consists of a first oligonucleotide being 10-50 nucleotides in length and comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 1: G GGC GCC ACT GCT AGA GA

SEQ ID 2: G TTC GGG CGC CAC TGC TAG A

25 SEQ ID 3: CGGGCGCCACTGCTA

and a second oligonucleotide being 10-50 nucleotides in length and comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 4: CTG CTT AAA GCC TCA ATA AA

SEQ ID 5: CTC AAT AAA GCT TGC CTT GA.

30 SEQ ID 12: GAT GCA TGC TCA ATA AAG CTT GCC TTG AGT

One of the oligonucleotides may serve as an "upstream oligonucleotide", i.e., an upstream-primer, while the second oligonucleotide serves as a "downstream oligonucleotide", i.e. downstream primer, in the amplification reaction. The location on the HIV-genome (or the sequence complementary thereto) to which both oligonucleotides comprised in such a pair

35 according to the invention can anneal, will together define the sequence of the nucleic acid that is amplified. The amplified sequence is located between the "primer-binding sites" within

the LTR region of the HIV-genome. It has been found that, by using a pair of oligonucleotides according to the invention in an amplification reaction, accurate and reliable amplification of nucleic acid derived from all presently known sub-types of HIV can be achieved.

- 5 A most preferred pair of oligonucleotides according to the invention will consist of a first primer comprising the sequence of SEQ ID NO 1 and a second primer with the sequence of SEQ ID NO 5. For use in a transcription based amplification method, the oligonucleotide with SEQ ID NO 9 is preferred, in combination with an oligonucleotide with the sequence of SEQ ID NO 5.
- 10 Part of the oligonucleotides according to the invention are particularly suitable for use as a probe in the detection of nucleic acid amplified with a pair of oligonucleotides according to the invention. When used as a probe, said oligonucleotides may be provided with a detectable label. Oligonucleotides according to the invention that are especially suitable as a probe consist essentially of the sequence
- 15 SEQ ID 6: TCT GGT AAC TAG AGA TCC CTC
SEQ ID 7: TAG TGT GTG CCC GTC TGT or
SEQ ID 8: AGT GTG TGC CCG TCT GTT
provided with a detectable label. A most preferred oligonucleotide in this respect is an oligonucleotide with a sequence as depicted in SEQ ID 6.
- 20 Various labeling moieties are known in the art. Said moiety may, for example, either be a radioactive compound, a detectable enzyme (e.g. horse radish peroxidase (HRP)), a hapten like biotin, or any other moiety capable of generating a detectable signal such as a colorimetric, fluorescent, chemiluminescent or electrochemiluminescent signal.
Hybrids between oligonucleotides according to the invention and (amplified) target nucleic
- 25 acid may also be detected by other methods known to those skilled in the art.
Evidently methods for amplification of nucleic acid, like the ones that have been mentioned above, using the oligonucleotides according to the present invention are also part of the invention.
The present invention further provides test kits for the amplification and detection of HIV
- 30 nucleic acid. The use of said test-kits enables accurate and sensitive screening of samples suspected of containing HIV derived nucleic acid. Such test-kits may contain a pair of oligonucleotides according to the invention and optionally also a oligonucleotide according to the invention that can be used as a probe for the detection of the amplified material.
Furthermore the test-kit may contain suitable amplification reagents. These reagents are for
- 35 example the suitable enzymes for carrying out the amplification reaction. A kit, adapted for use with NASBA, for example may contain suitable amounts of reverse transcriptase, RNase

H and T7 RNA polymerase. Said enzymes may be present in the kit in a buffered solution but can likewise be provided as a lyophilized composition, for example, a lyophilized spherical particle. Such lyophilized particles have been disclosed in PCT appl. no. EP95/01268. The kit may further be furnished with buffer compositions, suitable for carrying out an amplification reaction. Said buffers may be optimized for the particular amplification technique for which the kit is intended as well as for use with the particular oligonucleotides that are provided with the kit. In transcription based amplification techniques, such as NASBA, said buffers may contain, for example, DMSO, which enhances the amplification reaction (as is disclosed in PCT appl. no. US90/04733).

5 Furthermore the kit may be provided with an internal control as a check on the amplification procedure and to prevent the occurrence of false negative test results due to failures in the amplification procedure. The use of internal controls in transcription based amplification techniques is described in PCT appl. no. EP 93/02248. An optimal control sequence is selected in such a way that it will not compete with the target nucleic acid in the amplification reaction. Kits may also contain reagents for the isolation of nucleic acid from biological specimen prior to amplification. A suitable method for the isolation of nucleic acid is disclosed in EP389063.

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BRIEF DESCRIPTION OF THE FIGURES:

Figure1:

Amplification products after blotting and detection by enhanced chemiluminescence obtained after amplification in presence (panel A) or absence (panel B) of HIV-1 RNA using the following primer pairs. Lane1: SEQ ID 9-SEQ ID 10, lane 2: SEQ ID 9-SEQ ID 5, lane 3: SEQ ID 10-SEQ ID 4, lane 4: SEQ ID 10-SEQ ID 5, lane 5: SEQ ID 3-SEQ ID 12.

EXAMPLES

Example 1: Amplification and detection of HIV-1 genomic RNA.

30 The following procedures were applied to amplify and detect HIV-1 genomic RNA from samples as described in the following examples.

Sample preparation and nucleic acid isolation.

35 The isolation of the nucleic acids was performed according to Boom et al., 1990, Journal of Clinical Microbiology 28, 495-503 and European patent no. EP0389063. In short: A volume of 200 µl of pooled plasma's from healthy donors (negative for HBsAg, anti-HIV and anti-

HCV) was added to 900 μ l of lysis buffer (47 mM Tris-HCl pH 7.2, 20 mM EDTA, 1.2 % Triton X-100, 4.7 M guanidine thiocyanate (GuSCN, Fluka, Buchs, Switzerland)). After vortexing and centrifugation (30 seconds, 13,000 rpm) a dilution series of HIV-1 RNA subtype B standard, characterised and described by Layne et al., 1992, *Virology* 189, 695-

5 714 or HIV-1 positive specimens, was added. Upon addition of 50 μ l activated silicon (0.4 g/ml suspension in 0.1 N HCl), the suspension was incubated for 10 minutes at room temperature with regular vortexing. After centrifugation (30 - 60 seconds, 13,000 rpm) the silicon pellet was washed twice with 1 ml of wash buffer (5.25 M GuSCN, 50 mM Tris-HCl pH 6.4), followed by two 70% ethanol washes and one acetone wash step. Subsequently, the 10 silicon pellets were dried during ten minutes in a 56°C heating block. The nucleic acids were eluted from the silicon by adding 50 μ l elution buffer (1.0 mM Tris-HCl, pH 8.5) and incubation at 56°C for 10 minutes. Subsequently, 5 μ l of the eluate was taken of the pellet for further use in the amplification reaction. The remaining eluate was stored at -70°C.

15 NASBA amplification.

Amplifications were carried out in a reaction volume of 20 μ l which is composed of 10 μ l primer mixture, 5 μ l (isolated) nucleic acids and 5 μ l enzyme mixture. The primer mixture was made by reconstitution of a lyophilised accusphere into 50 μ l accusphere diluent, 51.6 μ l water, 8.4 μ l 2 M KCl and 5 μ l of each primer (10 μ M). The mixture was thoroughly vortexed before use. From this primer mixture 10 μ l was added to 5 μ l of the (isolated) HIV-1 RNA (standard). This mixture was incubated for 5 minutes at 65°C and subsequently incubated at 20 41°C for another 5 minutes. After these incubations 5 μ l enzyme mixture was added and incubated for 5 minutes at 41°C. The tubes were transferred to the detection area and incubated for 90 minutes at 41°C. After the amplification reaction the tubes were stored at - 25 20°C until further use.

Each amplification reaction contained the following reagents:

40 mM Tris-HCl, pH 8.5
12 mM MgCl₂
30 70 mM KCl
15 % v/v dimethyl sulfoxide
5 mM dithiotreitol
1 mM of each deoxynucleoside triphosphate
2 mM of the nucleosides rATP, rCTP, rUTP
35 1.5 mM rGTP
0.5 mM ITP

0.105 µg/ml BSA
0.08 units RNaseH
32 units T₇ RNA polymerase
6.4 units avian myeloblastosis virus reverse transcriptase (Seikagaku, USA)
5 0.2 µM of each primer
375 mM sorbitol
45.6 mM sucrose
28.5 mM mannitol
0.13 mM dextran T40
10

Detection of the amplified products.

A. Gel electrophoresis.

The presence of amplified products was analysed using an agarose gel (100 ml of 2 % Pronarose and 0.5 µg/ml ethidium bromide) and 1* TAE (40 mM Tris-acetate, 1 mM EDTA pH 8.0) running buffer are used. Electrophoresis was carried out at 100 volts for approximately 30 minutes. The ethidium bromide-stained bands of the amplified products were visualised using UV irradiation. The blot was hybridised with biotin probe (3 µM) in hybridisation mix (750 mM NaCl, 75 mM sodiumcitrate, 20 mM Na₂HPO₄/NaH₂PO₄ (pH 6.7), 10 * Denhardt's) by incubating the blot for 4 hours at 50°C. After hybridisation the blot was washed two times for 5 minutes at 50°C with 450 mM NaCl, 45 mM sodiumcitrate pH 6.4 (2 * SSPE) and 1% sodium dodecyl sulphate (SDS) solution and one time for 10 minutes with 20 mM Na₂HPO₄ pH 7.4, 360 mM NaCl, 2 mM EDTA and 0.1% SDS at room temperature. Subsequently, the blot was incubated for 30 minutes with 2 µl streptavidin/horse radish peroxidase solution (500 U/ml from the enhanced chemiluminiscense detection kit of Amersham Life Science) in 10 ml 50 mM Na₂HPO₄, 900 mM NaCl, 5 mM EDTA, pH7.4 and 0.5% SDS. Following washes respectively two times for 5 minutes in 2*SSPE, 0.1% SDS and once for 10 minutes in 2*SSPE at room temperature the blot was dried between tissues, developed and exposed to a film according to the Amersham kit protocol.

B. Electrochemiluminescence (ECL) probe hybridisation.

The amplification products were diluted two times in detection diluent (1.0 mM Tris/HCl, pH 8.5 and 0.2 g/l 2-methylisothiazolone HCl). Subsequently, 5 µl of the diluted amplification product was incubated for 30 minutes at 41°C with 0.084 µM of the HIV-1 specific biotin probe bound to 5 µg streptavidin-coated magnetic beads (mean size 2.8 µm ± 0.2 µm, Dynal, Great Neck, NY, USA) and 2*10¹¹ molecules of an ECL (Tris[2,2-bipyridine] ruthenium

[II] complex)-labelled probe, in a total volume of 25 μ l 750 mM NaCl, 75 mM sodiumcitrate, pH 6.4 (5 * SSC). As negative controls, detection diluent was also incubated with the bead-probe and ECL-probe mixtures. During incubation, tubes were agitated every 10 minutes to keep the beads in suspension. Subsequently, 300 μ l of assay buffer solution (100 mM 5 tripropylamine, pH 7.5) was added and the tubes were placed in an ECL detection instrument (NASBA QR-system from Organon Teknika BV) for reading the emitted ECL signals.

10 Example 2: Amplification and detection of HIV-1 genomic RNA.

The following primer pairs were tested on 10^4 copies (as determined by spectrophotometry at 260 nm) of the HIV-1 RNA standard. The RNA was added directly into the amplification. The analysis of the amplified products was performed by gel electrophoresis as described in 15 example 1. The results are shown in the figure 1.

All primer pairs and probes of the present invention were able to amplify and detect HIV-1 RNA from subtype B to a similar extent. Analytical sensitivity is shown for two primer pairs (SEQ ID 9/SEQ ID 5 and SEQ ID11/SEQ ID 5) using a dilution series of the HIV-1 RNA standard which has an initial concentration of $5.5 \cdot 10^9$ copies/ml. The detection was done 20 with probes having the sequences as depicted in SEQ ID 7 and SEQ ID 6. The amplification and detection was carried out as described in example 1. Table 1 shows the results obtained with both primer pairs.

Table 1.

HIV-1 RNA copies	SEQ ID 3-SEQ ID 5		SEQ ID 9-SEQ ID 5	
	No. detected	% detected	No. detected	% detected
200	4:4	100 %	4:4	100 %
100	8:8	100 %	8:8	100 %
50	8:8	100 %	8:8	100 %
25	7:8	87.5 %	7:8	87.5 %
12	6:8	75 %	4:8	50 %
6	2:8	25 %	4:7	57 %
3	0:8	0 %	2:7	28.5 %
0	0:8	0 %	0:7	0 %

Both primer pairs have approximately the same sensitivity for the HIV-1 RNA standard respectively a detection rate of 70% for the primer pair SEQ ID 9/SEQ ID 5 and a detection rate of 63% for the primer pair SEQ ID 3/SEQ ID 5.

Example 3: Amplification and detection of HIV-1 RNA in presence of an internal control.

10 In this example the primer pair SEQ ID 9/SEQ ID 5 and probe with the sequence of SEQ ID 7 was tested on dilution series of the HIV-1 RNA standard in presence of an internal control (ic-) RNA. The ic-RNA is an in vitro transcript which contains part of the LTR sequence of HIV-1 HXB-2 and 10^4 copies (as determined by spectrophotometry at 260 nm) are added prior to nucleic acids isolation. The isolation, amplification and ECL detection was performed

15 as described in example 1. As a comparison, separate amplification and detection was performed using a primer pair located in the HIV-1 gag region (P1/P2) previously described by Van Gemen et al, 1993, Journal of Virological Methods 43, 177-188. The probes used to detect the amplification products generated by the gag based primer pair were:

20 Biotin probe: 5'-TGTAAAAGAGACCATCAATGAGGA.
 ECL probe: 5'-GAATGGGATAGAGTGCATCCAGTG.

Results are shown in Table 2.

Table 2.

HIV-1 RNA copies/200µl	LTR SEQ ID 9-SEQ ID 5		GAG P1/P2	
	No. detected	% detected	No. detected	% detected
320	9:10	90 %	nt*	nt*
160	5:10	50 %	8:10	80 %
80	5:10	50 %	5:10	50 %
40	3:8	38 %	4:10	40 %
20	3:10	32 %	1:10	10 %
10	1:10	10 %	2:10	20 %
0	0:5	0 %	1:5	20 %

*nt is not tested.

Both the LTR primer pair of this example and the gag primer pair were able to detect a

5 similar amount of HIV-1 RNA in an assay controlled by an in vitro produced RNA which was added prior to the nucleic acids isolation.

Example 4: Detection of HIV-1 RNA in HIV-1 positive samples.

10

In this example, 40 HIV-1 positive samples from various geographical locations were analysed for presence of HIV-1 RNA. All samples were isolated by the nucleic acids isolation method described in example 1. Amplification and detection were carried out as described in example 1 using primer pair SEQ ID 9/SEQ ID 5 and probe with the sequence of SEQ ID 7 15 or probe with the sequence of SEQ ID 8.

For comparison, separate amplification and detection was carried out using the gag primer pair and probes as described in example 3. Presence of amplified products was detected by ECL probe hybridisation according to the method of example 1. Result are shown in Table 3.

Table 3.

Primer pair	ECL Probe	No. Detected	% Detected.
SEQ ID 9/SEQ ID 5 (LTR)	SEQ ID 7	40/40	100 %
SEQ ID 9/SEQ ID 5 (LTR)	SEQ ID 8	10/10	100 %
P1/P2 (gag)		29/40	72.5 %

Both primer probe combinations derived from the LTR region of HIV-1 described in this example were able to detect HIV-1 from all the samples tested. In contrast, the primer probe 5 combination of the gag assay failed to detect HIV-1 genomic RNA from a great number of samples.

Example 5: Amplification and detection of HIV-1 RNA with defined subtypes.

10 In this example, 33 samples harbouring HIV-1 RNA of known envelope-based subtypes were tested. The samples originate from subtyped viruses propagated in cell cultures. Samples of cell culture supernatants from both variants of group M subtypes A through H and variants of group O were spiked to HIV-1 negative human plasma and treated according to the method 15 from example 1. Amplification and detection was performed essentially as example 4. Results are shown table 4 as the number of samples of each type from which HIV-1 RNA was detected out of the total number of each type tested.

Table 4.

Primer pair	HIV-1 Types									
	A	B	C	D	E	F	G	H	O	Total
SEQ ID 9/SEQ ID 5 (LTR)	5:5	2:2	2:2	5:5	7:7	1:1	1:1	1:1	9:9	33:33
	4:5	2:2	2:2	5:5	6:7	1:1	1:1	1:1	0:9	22:33

20 HIV-1 RNA was detected from all 33 HIV-1 samples using LTR primer pair SEQ ID 9/SEQ ID 5 and probe with sequence of SEQ ID 7. In contrast, the assay using the gag primers probes combination as described in example 3 failed to detect subtype A and subtype E each from one of the samples and all samples containing HIV-1 RNA from group O members.

Example 6: Amplification and detection of HIV-1 clinical samples.

5 In this example, 7 samples obtained from seropositive patients originating from Africa, South America and Asia were assayed for the presence of HIV-1 genomic RNA. Despite the fact that blood samples from these patients are both anti-p24 antibody (Abbott Laboratories, Abbott Park, IL) and western blot (Genelabs) positive, all specimens were scored negative by the NASBA HIV-1 RNA QL assay (Organon Teknika BV) which uses the gag primer pair
10 and probe combination from example 3 and only a single sample (R9612222) was detected and quantitated (29.500 RNA copies/ml) by the Quantiplex HIV-1 RNA 2.0 (Chiron) assay using a sample volume of 50 µl. In contrast, using an equal sample volume the primer pair SEQ ID 9/SEQ ID 5 and the probe with sequence of SEQ ID 7 detected four out of seven samples (Table 5).

15

Table 5.

Sample code	Country	SEQ ID 9/SEQ ID 5 (LTR)
R9610155	Thailand	positive
R9612222	Ghana	positive
R9700062	Brazil	negative
R9612218	Zaire	negative
R9611710	Liberia	positive
R9610718	Antilles	positive
R9607884	Rwanda	negative

20 The samples missed by the LTR primer probe combination are not detected due to a low viral load below the detection level since by using a sample volume of one ml the Quantiplex HIV-1 RNA 2.0 (Chiron) assay quantitated HIV-1 RNA levels of 30 or below 30 HIV-1 RNA copies per 50 µl of these samples.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

- (A) NAME: AKZO NOBEL N.V.
- (B) STREET: Velperweg 76
- (C) CITY: Arnhem
- 10 (E) COUNTRY: The Netherlands
- (F) POSTAL CODE (ZIP): 6824 BM

(ii) TITLE OF INVENTION: Nucleic acid sequences that can be used as
primers and probes in the amplification and detection of
15 all subtypes of HIV-1.

(iii) NUMBER OF SEQUENCES: 12

(iv) COMPUTER READABLE FORM:

- 20 (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

25

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

40 GGGCGCCACT GCTAGAGA

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

15 GTTCGGGCGC CACTGCTAGA

20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CGGGCGCCAC TGCTA

15

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40

5 (ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

10 CTGCTTAAAG CCTCAATAAA

20

10 (2) INFORMATION FOR SEQ ID NO: 5:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

25 CTCATAAAAG CTTGCCTTGA

20

25 (2) INFORMATION FOR SEQ ID NO: 6:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

40 TCTGGTAAC AGAGATCCCT C

21

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

15 TAGTGTGTGC CCGTCTGT

18

(2) INFORMATION FOR SEQ ID NO: 8:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AGTGTGTGCC CGTCTGTT

18

(2) INFORMATION FOR SEQ ID NO: 9:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40

5 (ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AATTCTAATA CGACTCACTA TAGGGAGAGG GGCGCCACTG CTAGAGA

47

10 (2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AATTCTAATA CGACTCACTA TAGGGAGAGG TTGGGGCGCC ACTGCTAGA

49

25

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- 30 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

40 AATTCTAATA CGACTCACTA TAGGGCGGGC GCCACTGCTA

40

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

15 GATGCATGCT CAATAAAGCT TGCCTTGAGT

30

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CLAIMS:

1. Pair of oligonucleotides, for use as a set in the amplification of a target sequence located within the LTR region of the genome of HIV-1, said pair consisting of a first oligonucleotide being 10-50 nucleotides in length and comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 1: G GGC GCC ACT GCT AGA GA

SEQ ID 2: G TTC GGG CGC CAC TGC TAG A

SEQ ID 3: CGGGCGCCACTGCTA

and a second oligonucleotide being 10-50 nucleotides in length and comprising, at least a fragment of 10 nucleotides, of a sequence selected from the group consisting of:

SEQ ID 4: CTG CTT AAA GCC TCA ATA AA

SEQ ID 5: CTC AAT AAA GCT TGC CTT GA.

SEQ ID 12: GAT GCA TGC TCA ATA AAG CTT GCC TTG AGT

2. Pair of oligonucleotides according to claim 1, consisting of a first oligonucleotide being 10-50 nucleotides in length and comprising, at least a fragment of 10 nucleotides of the sequence

SEQ ID 1: G GGC GCC ACT GCT AGA GA and a second oligonucleotide being 10-50 nucleotides in length and comprising, at least a fragment of 10 nucleotides of the sequence

SEQ ID 5: CTC AAT AAA GCT TGC CTT GA.

3. Pair of oligonucleotides according to claim 1 or 2 wherein the first oligonucleotide is provided with a promoter sequence recognized by a DNA dependent RNA polymerase.

4. Pair of oligonucleotides according to claim 3 consisting of a first oligonucleotide consisting essentially of the sequence

SEQ ID 9: *aat tct aat acg act cac tat agg gAG AGG GGC GCC ACT GCT AGA*

GA and a second oligonucleotide consisting essentially of the sequence

SEQ ID 5: CTC AAT AAA GCT TGC CTT GA.

5. Method for the detection of HIV-1 nucleic acid in a sample wherein the sample is subjected to a nucleic acid amplification reaction using a pair of oligonucleotides according to any of claims 1-4 and suitable amplification reagents and the presence of any amplified nucleic acid is detected.

Amend

6. Method according to claim 5, wherein the detection of any amplified nucleic acid is carried out by reacting the sample with one or more oligonucleotides having (a) sequence(s) selected from the group: consisting of:

SEQ ID 6: TCT GGT AAC TAG AGA TCC CTC

SEQ ID 7: TAG TGT GTG CCC GTC TGT or

SEQ ID 8: AGT GTG TGC CCG TCT GTT

or more of which are provided with a detectable label under suitable hybridization conditions and detecting the presence of the label in any hybrids formed between the amplified sequence and the probe.

7. Method according to claim 5, wherein the amplification technique used is a transcription based amplification technique.

8. Test kit for the detection of HIV-1 in a sample comprising:

- a pair of oligonucleotides according to claim 1,
- one or more oligonucleotides comprising a nucleic acid sequence substantially complementary to at least part of the amplified nucleic acid sequence, provided with a detectable label
- suitable amplification reagents.

9. Test kit for the detection of HIV-1 in a sample comprising a pair of oligonucleotide according to claim 4, together with suitable amplification reagents and means for detecting the amplified nucleic acid.

10. Use of a pair of oligonucleotides as a set of primers in the amplification of a target sequence located within the LTR region of the genome of HIV-1.

Amplification products after blotting and detection by enhanced chemiluminiscence.

A

1 2 3 4 5



B

1 2 3 4 5



FIGURE 1

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

T/97300 65

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original first and joint inventor (if plural names are listed below) of the subject matter for which a patent is sought on the invention entitled:

"NUCLEIC ACID SEQUENCES THAT CAN BE USED AS PRIMERS AND PROBES IN THE AMPLIFICATION AND DETECTION OF ALL SUBTYPES OF HIV-1"

the specification of which

[CHECK ONE]

[] is attached hereto

[] was filed on _____ as Application Serial No.

and was amended on _____

[if applicable]

[X] as filed under the Patent Cooperation Treaty on August 05, 1998
Serial PCT/EP98/04945 The United States of America being designated.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined Title 37, Code of Federal Regulations Section 1.56(a)

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign applications(s) for patent or inventor's certificate having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)		Priority claimed		
Number	Country	Day/Month/Year filed	<input checked="" type="checkbox"/> Yes	No
97202455.8	EP	08 / 08 / 1997		
Number	Country	/ /	Yes	No
Number	Country	Day/Month/Year filed		
Number	Country	/ /	Yes	No
		Day/Month/Year filed		

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the patent and Trademark

Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which

became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

(U.S. Serial No.) (Filing date) (Status-patented, pending, abandoned)

(U.S. Serial No.) (Filing date) (Status-patented, pending, abandoned)

And I hereby appoint as principal attorney, William M. Blackstone, Registration No. 29,772, Mary E. Gormley, Registration No. 34,409, Gregory R. Muir, Registration No. 35,293 and Michael G. Sullivan, Registration No. 35,377.

Please address all communications to:

William M. Blackstone
AKZO NOBEL
1300 Piccard Drive #206
Rockville, MD 20850-4373

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Jaap GOUDSMIT

Inventor's signature _____ Date _____

Citizenship _____ Dutch _____

Residence and P.O. Address Koninginneweg 4, 1075 CX AMSTERDAM - The Netherlands

Full name of second joint inventor Pieter OUDSHOORN

Inventor's signature P. O. J. Y. 22-12-99 Date _____

Citizenship _____ Dutch _____

Residence and P.O. Address Wilgenstraat 6, 5271 JH St. MICHELSGESTEL - The Netherlands NLX

Full name of third joint inventor Suzanne JURRIAANS

Inventor's signature _____ Date _____

Citizenship _____ Dutch _____

Residence and P.O. Address Speet 33, 1141 BX MONICKENDAM - The Netherlands

Full name of forth joint inventor Vladimir, Vladimirovich LUKASHOV

Inventor's signature _____ Date _____

Citizenship _____ Russian _____

Residence and P.O. Address Eeftink 621, 1103 AE AMSTERDAM - The Netherlands

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original first and joint inventor (if plural names are listed below) of the subject matter for which a patent is sought on the invention entitled:

"NUCLEIC ACID SEQUENCES THAT CAN BE USED AS PRIMERS AND PROBES IN THE AMPLIFICATION AND DETECTION OF ALL SUBTYPES OF HIV-1"

the specification of which

[CHECK ONE]

[] is attached hereto

[] was filed on _____ as Application Serial No.

and was amended on _____

[if applicable]

[X] as filed under the Patent Cooperation Treaty on August 05, 1998
Serial PCT/EP98/04945 The United States of America being designated.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

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I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign applications(s) for patent or inventor's certificate having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)		Priority claimed
<u>97202455.8</u>	<u>EP</u>	<u>08 / 08 / 1997</u>
Number	Country	Day/Month/Year filed
_____	_____	/ /
Number	Country	Day/Month/Year filed
_____	_____	/ /
Number	Country	Day/Month/Year filed

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the patent and Trademark

Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which

became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

(U.S. Serial No.) (Filing date) (Status-patented, pending, abandoned)

(U.S. Serial No.) (Filing date) (Status-patented, pending, abandoned)

And I hereby appoint as principal attorney, William M. Blackstone, Registration No. 29,772, Mary E. Gormley, Registration No. 34,409, Gregory R. Muir, Registration No. 35,293 and Michael G. Sullivan, Registration No. 35,377.

Please address all communications to:

William M. Blackstone
AKZO NOBEL
1300 Piccard Drive #206
Rockville, MD 20850-4373

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

100 Full name of sole or first inventor Jaap GOUDSMIT

Inventor's signature  Date

Citizenship Dutch 24-12-99

Residence and P.O. Address Koninginneweg 4, 1075 CX AMSTERDAM - The Netherlands NLX

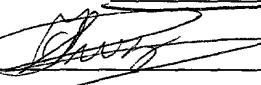
Full name of second joint inventor Pieter OUDSHOORN

Inventor's signature _____ Date

Citizenship Dutch

Residence and P.O. Address Wilgenstraat 6, 5271 JH St. MICHELSGESTEL - The Netherlands

300 Full name of third joint inventor Suzanne JURRIAANS

Inventor's signature  24-12-99 Date

Citizenship Dutch

Residence and P.O. Address Bereklauw 17 KK
Sloop 33, 1141 BX MONICKENDAM - The Netherlands NLX

400 Full name of forth joint inventor Vladimir Vladimirovich LUKASHOV

Inventor's signature  24-12-99 Date

Citizenship Russian

Residence and P.O. Address Waterkant 25, 1112 RB Diemen
Eefink 621, 1103 AE AMSTERDAM - The Netherlands NLX

09/463352

420 Rec'd PCT/PTO 21 JAN 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

GOUDSMIT ET AL.

Serial Number: to be assigned

Group Art Unit: to be assigned

Filed: concurrently herewith

Examiner: to be assigned

For: NUCLEIC ACID SEQUENCES THAT CAN BE USED AS PRIMERS AND PROBES IN THE AMPLIFICATION AND DETECTION OF ALL SUBTYPES OF HIV-1

SUBMISSION OF SEQUENCE LISTING

Assistant Commissioner of Patents
Washington, D.C. 20231

January 21, 2000

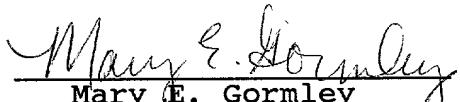
Sir:

Applicants are submitting herewith the Sequence Listing for the above-identified application both in paper copy form and in computer readable form.

The name of the file on the computer readable form is 97300us.app. The paper copy and the computer readable form are the same. No new matter is added hereby.

Please replace the current sequence listing in the specification with the instant sequence listing.

Respectfully submitted,


Mary E. Gormley
Attorney for Applicants
Registration No. 34,409

Attorney Docket # T/97300 US
AKZO NOBEL PHARMA
1300 Piccard Drive, Suite 206
Rockville, Maryland 20850
Tel: (301) 948-7400
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MEG:mg
10goudsmit.seq

Express Mail No. EL 24970404615

SEQUENCE LISTING

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Oudshoorn, Pieter
Jurriaans, Suzanne
Lukashov, Vladamir Vladamirovich

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